DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS IN SOME EDIBLE OILS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
AGAL	Australian Government Analytical Laboratories
AOAC	Association Official Analytical Chemistry
ATSDR	Agency for Toxic Substances and Disease Registry
BaP	Benzo(a)pyrene
CO_2	Carbon dioxide
EC	European Commission
EDS	Endocrine disrupting substance
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FAOSTAT	FAO statistics division
FDA	Food and Drug Administration
GC-ECD	Gas chromatograph - electon capture detector
GC-FID	Gas chromatograph - flame ionization detection
GC-FPD	Gas chromatograph - flame photometric detection
GPC	Gel permeation chromatography
IARC	International agency for research on cancer
IPEP	International POPs Elimination Project
IUPAC	International union of pure and applied chemistry
JECFA	Joint FAO/WHO Experts Committee on Food Additives
LC_{50}	Acute oral toxicity in fishes-lethal concentration 50
LD_{50}	Acute oral toxicity in rats-lethal dose 50
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDL	Method Detection Limit
MPOB	Malaysia Palm Oil Board

MRLs	Minimum risk levels
MRLs	Maximum residue limits
MS	Mass spectrometer
NaOH	Sodium hydroxide
NSM	Nutrition Society of Malaysia
OCP	Organochlorine pesticides
OPP	Organophosphorus pesticides
PAH	Polynuclear aromatic hydrocarbons
PAN	Pesticide Action Network
PANNA	Pesticide action network North America
PCBs	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzo-p-furans
POPs	Persistent Organic Pollutants
SFE	Supercritical-fluid extraction
SIM	Selective ion monitoring
SPE	Solid-phase extraction
SPME	Solid phase microextraction
TDS	Total diet study
TEF	Toxic equivalent factor
TEQ	Toxic equivalent quotient
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

PENENTUAN PENCEMAR ORGANIK PERSISTEN DALAM BEBERAPA MINYAK MASAK

Abstrak

Tahap kepekatan bagi 20 pestisid organoklorin (OCP), 8 pestisid organofosforus (OPP) dan 16 polinuklear aromatik hidrokarbon (PAH) telah dikaji dalam 8 sampel minyak masak. Kaedah analisis telah dibangunkan dan disahkan untuk menentukan kepekatan pencemar-pencemar ini dalam minyak masak tersebut. Sampel minyak tersebut pada mulanya diekstrak dengan menggunakan pelarut acetonitril, diikuti dengan kromatografi turus menggunakan florisil untuk memisahkan OCP dan OPP dari baki minyak; dan kromatografi turus silica gel untuk mengasingkan PAH dari matrik sampel. Analit-analit sasaran kemudiannya dianalisis dengan menggunakan GC dengan turus kapilari yang dilengkapi dengan pengesan pengionan nyalaan (FID) bagi OPP dan PAH; dan pengesan penangkapan elektron (ECD) bagi penentuan OCP. Eksperimen pemakuan perolehan semula menunjukkan keputusan perolehan semula yang baik bagi semua sebatian OCP, OPP dan PAH pada tiga tahap kepekatan yang berlainan (1, 5 dan 10 µg/g bagi OPP dan PAH dan 5, 20 dan 40 ng/g bagi OCP).

Kajian menunjukkan bahawa minyak kacang soya dan minyak lenga mempunyai kepekatan OCP yang tertinggi manakala minyak zaitun menunjukkan kepekatan OCP total yang terendah. Di antara dua puluh OCP yang dikaji, endosulfan dikesan di dalam kepekatan yang paling tinggi di dalam hampir semua jenis minyak masak yang dikaji sementara DDE dan dieldrin menunjukkan kepekatan yang paling rendah. Minyak zaitun dikesan mengandungi kepekatan tertinggi bagi kebanyakan sebatian OPP manakala minyak kacang soya mempunyai kepekatan paling rendah. Di antara lapan OPP yang dikaji, chlorpyrifos dan dichlorvos menunjukkan kepekatan yang tinggi di dalam kesemua jenis minyak masak, sementara parathion methyl menunjukkan kepekatan yang terendah. Tahap kepekatan bagi PAH dikesan paling

tinggi di dalam minyak biji bunga matahari manakala paling rendah di dalam minyak kacang soya. Antara 16 PAH yang dikaji, naftalena menunjukkan kepekatan yang paling tinggi di dalam semua minyak masak yang dikaji, manakala fluorena menunjukkan kepekatan yang paling rendah. Benzo(g,h,i)perylena tidak dikesan di dalam mana-mana sampel minyak masak tersebut.

Dalam tesis ini, kesan memasak makanan pada suhu yang tinggi juga dapat dilihat. Selepas sampel minyak dipanaskan pada suhu 200 °C selama 2 jam, minyak kanola, minyak beras dan minyak zaitun menunjukkan peningkatan drastik kepekatan PAH total, manakala minyak sawit menunjukkan penurunan kepekatan PAH. Anthracena menunjukkan peningkatan kepekatan di dalam beberapa sampel minyak selepas dipanaskan. Benzo(g,h,i)perylena yang tidak dikesan dalam mana-mana sampel minyak masak sebelum ianya dipanaskan, walaubagaimanapun, terhasil selepas ianya dipanaskan.

Keputusan tahap kepekatan yang diperoleh di atas kemudiannya digunakan untuk mengira pengambilan diet harian anggaran bagi sisa OCP dan OPP melalui pemakanan minyak masak oleh warga Malaysia. Pengambilan diet harian anggaran tersebut kemudiannya dibandingkan dengan pengambilan harian yang dibenarkan (ADI) yang ditetapkan oleh Badan Kesihatan Sedunia (WHO), yang merupakan tahap selamat racun perosak dalam makanan, atau tahap kepekatan berisiko rendah (MRLs) oleh badan-badan lain. Jika dibandingkan dengan anggaran pengambilan harian yang dilaporkan di dalam laporan rujukan, keputusan yang diperolehi di dalam kajian ini secara umumnya jauh lebih rendah daripada laporan-laporan tersebut. Ini mencadangkan bahawa minyak masak yang terdapat di Malaysia adalah selamat untuk dimakan.

DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS IN SOME EDIBLE OILS

Abstract

The concentration levels of 20 organochlorine pesticides (OCP), 8 organophosphorus pesticides (OPP) and 16 polynuclear aromatic hydrocarbons (PAH) had been investigated in 8 edible oil samples. Analytical methods were developed and validated to determine the concentrations of these pollutants in edible oils. The oil sample was first extracted using acetonitrile, followed by florisil column chromatography to separate OCPs and OPPs from residual oil; and silica gel column chromatography to isolate PAHs from sample matrices. Target analytes were analysed using GC capillary column equipped with flame ionization detector (FID) for OPPs and PAHs; and electron capture detector (ECD) for OCPs determination. Spiked recovery experiments showed good recoveries of all OCPs, OPPs and PAHs compounds at three different concentration levels (1, 5 and 10 µg/g for OPP and PAH; and 5, 20 and 40 ng/g for OCP) respectively.

The study found that soybean oil and sesame oil had the highest concentration of OCPs while olive oil showed the lowest concentration of total OCPs. Among the twenty OCPs studied, endosulfan was found to be the highest in concentration in almost all edible oils studied while DDE and dieldrin showed the lowest concentrations. Olive oil was found to contain the highest concentration of most OPP compounds, while soybean oil showed the lowest concentration. Among the eight OPPs studied, chlorpyrifos and dichlorvos were found to be high in concentrations in all edible oils studied, while parathion methyl showed the lowest concentration. PAHs levels were found the highest in sunflower seed oil and the lowest in soybean oil. Among 16 PAHs studied, naphthalene showed the highest concentration in all edible oils studied, while fluorene showed the lowest concentration. Benzo(g,h,i)perylene was not detected in any of the oil samples.

In this thesis, the effect of cooking food at high temperature has been studied. It was found that after the oil samples were heated at 200 °C for 2 hours, canola oil, rice bran oil and olive oil showed drastic increase in total PAH concentration, while palm oil showed decrease in PAH concentration. Anthracene was found to increase after heating in several oil samples. Benzo(g,h,i)perylene which was not found in any of the unheated oil was however detected after the oil samples were heated.

The concentration level results were then used to calculate the estimated dietary intake of OCP and OPP residue through consumption of edible oils by Malaysian population. The estimated dietary intake is then compared against the Acceptable Daily Intake (ADI) regulated by World Health Organization (WHO), which represents an acceptable safe level of consuming the pesticides, or Minimal Risk Levels (MRLs) by other organization. In comparison with the estimation of daily intake reported in the literature, the results from this study were generally lower from those reports, which may suggests that the edible oils available in Malaysia are safe to be consumed.

CHAPTER 1

INTRODUCTION

1.0 Overview

Pesticides are widely used in crop production. The term pesticide includes ingredients used in products, such as insecticides, fungicides, rodenticides, insect repellants, weed killers and antimicrobials, which are designed to prevent, destroy, repel or reduce pests. The use of pesticides in food production has provided numerous benefits in terms of increasing production and quality. Unfortunately, consumers are exposed to pesticides usually through dietary intake of several food groups including fruits, juices, honey and vegetables.

Many pesticides may leave residues on treated fruits, vegetables, grains and other foods. Pesticide residues often decreased over time and as food crops are washed, stored, processed and prepared. However, some residues remain in both fresh produce such as corn and soybean and processed foods such as corn oil and soybean oil.

The studies of pesticide levels in edible oils are very limited in Malaysia. The Malaysian Palm Oil Board (MPOB) had reported a few studies on pesticide residue in palm oil, namely organochlorine pesticides, organophosphorus pesticides and paraquat (Halimah *et al*., 1999; Kuntom *et al*., 1999; Kuntom *et al*., 2000; and Halimah *et al*., 2004). However, the focus of the studies was on evaluating suitable methods for extracting these pesticides from palm oil matrix. They reported the efficiency of various extraction techniques such as liquid-liquid extraction, sweep co-distillation and gel permeation chromatography. Very limited data was reported on the actual concentration of pesticides in palm oil. Our project will study the level of pesticides in several edible oils including palm oil which are available in the market. The data will be used to estimate the health risk to the consumers on pesticides through dietary intake.

1.1 Objectives

The main objective of this study is to determine the concentration levels of pesticides in edible oil. It will be quite a challenge to isolate trace organic pollutants from organic matrices. Several studies had reported methods for isolating pesticides in oil with mixed success (Halimah *et al*., 1999; Rojo Camargo & Toledo, 2003; and Moret & Conte, 2000). Therefore, one of objective was to develop the method for the extraction of pesticides in edible oil and optimized the procedure for most efficient extraction. To achieve this objective, the study was designed as follows;

1. To develop and modify suitable method and validate the analytical methods for determination of organophosphorus pesticides (OPP), polynuclear aromatic hydrocarbons (PAH) and organochlorine pesticides (OCP) in oil matrix.
2. To determine the levels of OPP, PAH and OCP levels in several edible oils.
3. To investigate the effects of heating on the levels of PAH in these oils, and;
4. To estimate dietary intake of OCP and OPP through consumption of edible oils.

CHAPTER 2

LITERATURE SURVEY

2.1 Persistent Organic Pollutants (POPs)

POPs are chemical substances that are toxic and persistent in the environment. They can accumulate in the fatty tissues of living organisms and increase in concentration from one species to the next species higher in the food chain. Due to the high persistency, they can be transported through the atmosphere, oceans and other pathways, resulting in POPs pollution in remote area far from point of release.

There are twelve POPs that have been selected under Stockholm Convention in 2001. These include certain insecticides, such as aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB) and toxaphene; polychlorinated biphenyl (PCBs), mirex as well as certain byproducts such as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzo-*p*-furans (PCDF) (USEPA, 2002).

These compounds are used in various sectors. For example, PCBs are used in hundreds of commercial applications, such as in electrical, heat transfer and hydraulic equipment and as plasticizers in paints, plastics and rubber products, while PCDD and PCDF are produced unintentionally from most forms of combustion, including municipal and medical waste incinerators, open burning of trash and industrial processes (USEPA, 2002).

Out of the twelve chemicals targeted by the treaty, there are nine organochlorine pesticides; all of which have already been banned in the Malaysia (UNEP, 2002). The treaty is an international effort to phase out harmful chemicals that persist in the environment and can be transported around the world. Under this treaty, the join countries agree to reduce or eliminate the production, use or release of these 12 POPs. Table 2.1 shows status of POPs in Malaysia.

Table 2.1: Status of POPs in Malaysia (UNEP, 2002)

POPs	Status
Aldrin	Banned in 1994
Chlordane	Banned in 1998
DDT	Banned in 1999
Dieldrin	Banned in 1994
Endrin	Withdrawn by company
Heptachlor	Banned in 1990
HCB	Never registered
Mirex	Banned
Toxaphene	Banned

The exposure of these pollutants to humans can take place through skin absorption, respiration and especially ingestion of contaminated food (Safe, 1998), although unfortunately, some of them can take place through accidental contamination (Chen *et al*., 1982; Kannan *et al*., 1992) and at workplace (Nordin, *et al*., 2002). For example, in 1991, 893 cases of poisoning were reported in government hospitals throughout Malaysia, 278 (31.1%) of which were fatal (Adeel, 2003). Furthermore, accidental exposures at work accounted for about 11% of the 6554 poisoning cases reported between 1989 and 1994 (Mokhtar, 1996).

In recent years there has been increasing concern over environmental pollutants that can mimic estrogenic effects, often referred to as endocrine disruptors. Adverse effects from exposure to these chemicals arise due to interference of the chemical with natural hormones in the bodies of the affected organisms (Kavlock *et al*., 1996).

Reported adverse effects in wildlife include population declines, increases in cancers, reduced reproductive function, and disrupted development of immune and

nervous systems (Jimenez, 1997). In addition to environmental pollutants such as PCBs, bisphenols and dioxins, numerous studies have indicated that some pesticides also elicit toxic responses which resulted in disruptions of endocrine system (Adeel, 2003). These include DDT and its metabolites, aldicarb, atrazine, benomyl, endosulfan, lindane, malathion, mancozeb, maneb and permethrin amongst others (Gascon *et al*., 1997). Many of these chemicals are widely used in Malaysia, including in the paddy fields and palm oil plantations.

2.1.1 Organophosphorus pesticides (OPP)

Organophosphorus pesticides (OPP) are classified as insecticides; use to eradicate insects and undesirable pests. Most OPP are polar, water-soluble chemicals but a few lipophilic compounds are available and have been formulated in petroleum distillate vehicles. Table 2.2 shows structures of organophosphorus compounds monitored in this study.

This group of chemicals replaced the organochlorine pesticides banned for use in the United States in the 1970's. OPP accounts for about half of all insecticides used in the USA (USEPA, 1999). OPP are widely used in crops such as corn, cotton and wheat, canola and alfalfa, fruits and nuts; and vegetables which amount to 7-19 millions of pounds per year. Besides, OPP are also applied in non-agricultural sectors such as livestock and pet, grain storage as well as for turf and ornamental purposes. Some are also applied for residential and commercial uses such as termite control and mosquito control to protect public health against diseases such as malaria, dengue fever and encephalitis. These uses amount 1 to 4 millions pounds per year. Table 2.3 shows the uses of organophosphates pesticides in agricultural and nonagricultural purposes.

Table 2.2: OPP compounds monitored in this study

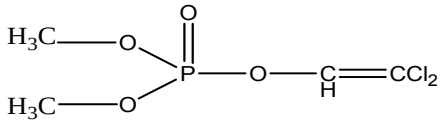
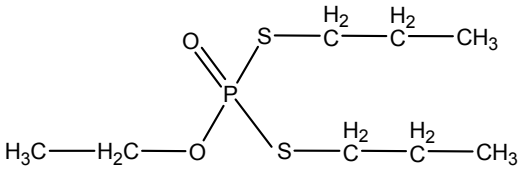
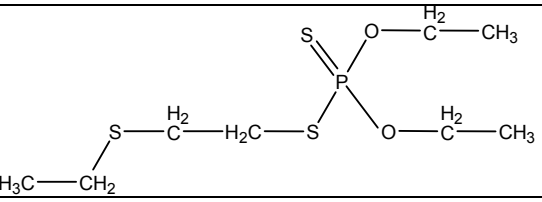
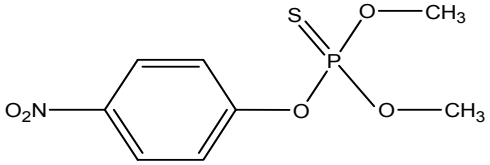
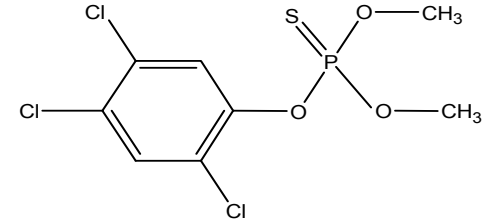
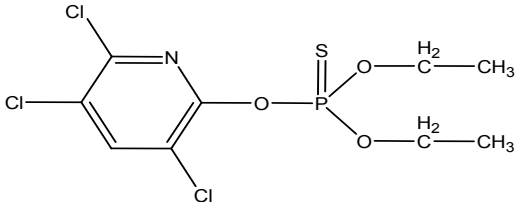
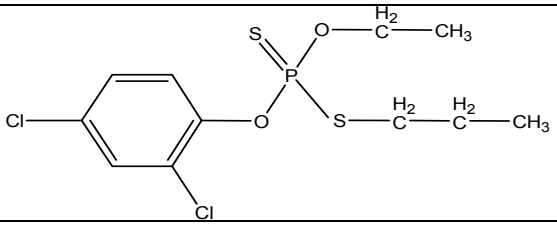
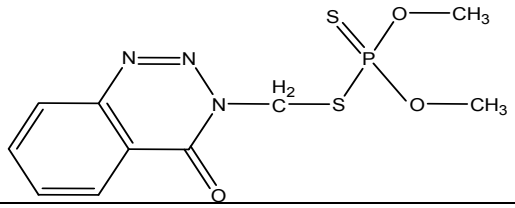
No	Compound	Structure
1.	Dichlorvos	
2.	Ethoprophos	
3.	Disulfoton	
4.	Parathion methyl	
5.	Fenchlorphos	
6.	Chlorpyrifos	
7.	Prothiofos	
8.	Guthion	

Table 2.3: Uses of organophosphorus pesticides (USEPA, 1999)

Agricultural uses of organophosphorus	***Nonagricultural uses of organophosphates***
• Field corn (19) • Cotton (15) • Other field crops, e.g., canola and alfalfa (10) • Fruits and nuts (9) • Vegetables (7) ( ) millions of pounds per year	• Livestock and pets (4) • Residential and commercial uses: termite control (4) and other (3) • Mosquito control and other uses (forestry, rangeland, etc.) (3) • Grain storage (2) • Turf/ornamental (1)

Approximately 60 million pounds of OPP were applied to 60 million acres of agricultural crops in the USA annually. Nonagricultural uses account for about 17 million pounds per year (USEPA, 1999).

The wide use of OPP is due to several factors such as inexpensive and broad spectrum properties as they can be used on several crops to control a variety of insect pests. In general, insects have not developed resistance to OPP as they have, to some other pesticides (USEPA, 1999).

OPP were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous and were used in World War II as nerve agents but they usually degrade rapidly in the environment and do not accumulate or concentrate in the biota. Thus, OPP have less potential for chronic health effects or environmental contamination then do organochlorine pesticides (OCP) and pose less risk to consumers through food intake.

OPP compounds, however, have higher acute toxicity to human. Most of these chemicals are classified by the U.S. Environmental Protection Agency as toxicity class I (highly toxic) or toxicity class II (moderately toxic). Some of the OPP are carcinogenic and known or suspected endocrine disruptors which can also cause developmental or reproductive harm (PANNA, 2004). Table 2.4 shows toxicity of OPP towards fishes and rats.

Table 2.4: Toxicity of OPP towards fishes and rats (Verschueren, 1983; PAN, 2005)

OPP	**LC_{50} (Fish) mg/L**	**LD_{50} (Rat, oral) mg/kg**	**Relative Toxicity**
Dichlorvos	0.87	56 – 80	Possible carcinogen
Ethoprophos	1.15	33 – 62	Carcinogen, extremely toxic
Disulfoton	3.70	2.6 -12.5	Highly toxic
Parathion methyl	0.20	3.6 – 13	Highly toxic
Fenchlorphos	0.31	1740	Slightly toxic
Chlorpyrifos	0.26	135 – 163	Suspected EDS
Prothiofos	2.00	925	Moderately toxic
Guthion	3.29	13 – 16.4	Highly toxic

Human are commonly exposed to OPP through eating fresh and processed vegetables, contacting pesticide-contaminated surfaces, breathing air near pesticide applications (both indoors and outdoors), and drinking pesticide contaminated water. The multiple uses and ubiquitous nature of these chemicals result in routine exposures to many different OPP for most human.

One of the important aspects for minimizing the potential hazards to human health is the monitoring of pesticide residues in food. The total diet study (TDS) or sometimes referred to as the “market basket” study, which was first initiated by the US

Food and Drug Administration (FDA), has been the method of choice for the monitoring of pesticide residue in food and the assessment of their intake by the population (Pennington, 2000; Sawaya *et al*., 2000). The collection of foods to be analyzed for pesticide residues in such study is based on the food consumption surveys, which constitutes the basis for selection of the diets mainly through food supply and food consumption patterns of the population.

For example, a “market basket” study of dietary components conducted by Sawaya *et al*. (2000) in Kuwait for 15 to 19 year old males and females revealed that seven OPP were detected in the food items. Chlorpyrifos methyl and fenitrothion were widely detected particularly in cereal and cereal products (mostly in grains), followed by the composite dishes, many of which contained flour or dough. Other OPP such as diazinon, chlorpyrifos, monocrotophos, dimethoate and omethoate, were less frequently detected and found mostly in fruits and vegetables.

2.1.2 Polynuclear Aromatic Hydrocarbons (PAH)

Polynuclear aromatic hydrocarbons (PAH) are a large group of organic compounds with two or more fused aromatic rings. They have relatively low solubility in water, but are highly lipophilic. Most of the PAH with low vapor pressures are adsorbed on particles and suspended in air. They can travel long distances before they return to earth in rainfall or particle settling.

PAH content of plants and animals living on land or in water can be many times higher than the content of PAH in soil. PAH can break down into longer-lasting products by reacting with sunlight and other chemicals in the air. Break down in soil and water generally takes weeks to months and is caused primarily by the actions of microorganisms (ATSDR, 1995).

PAH are formed mainly as a result of pyrolytic processes, especially the incomplete combustion of organic materials during industrial and other human activities, such as processing of coal and crude oil, combustion of natural gas,

combustion of refuse, motor-vehicle, cooking and tobacco smoking as well as in natural processes such as carbonization. A few of them are used in medicines and to make dyes, plastics and pesticides. For example, naphthalene is used in making dyes, explosives, plastics, and lubricants; and moth repellent while anthracene is used in dyes, insecticides and wood preservatives.

There are more than 100 PAH found in nature and 17 have been selected as priority pollutants by the United States Environmental Protection Agency (EPA) on the basis of their occurrence and carcinogenicity (ASTDR, 1995). The PAH compounds monitored in this study are shown in Figure 2.1. Table 2.5 shows summary of short-term toxicity tests following oral administration for a number of PAH.

A number of techniques have been developed for the biological monitoring of human exposures to PAH. Benzo(a)pyrene (BaP) has frequently been used as indicator of PAH toxicity in the environment since it has been known as the most carcinogenic PAH (WHO, 2000). Table 2.6 shows sources of BaP and routes of exposures.

Food can be contaminated by environmental PAH that are present in air (by deposition), soil (by transfer) or water (by deposition and transfer), or during processing and cooking. In areas remote from urban or industrial activities, the levels of PAH found in unprocessed foods reflects the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. In the neighborhood of industrial areas or along highways, the contamination of vegetation can be ten-fold higher than in rural areas (Larsson and Sahlberg, 1982).

Most people are exposed to PAH through the food they eat, depending on the mode of cooking, preservation and storage, especially food that have been grilled, barbequed or smoked. PAH were detected in a wide range of meats, fishes, vegetable and fruits. According to Menzie *et al*. (1992), the general population ingests 1-9 µg of PAH per day through consumption of food, making food the primary route of exposure.

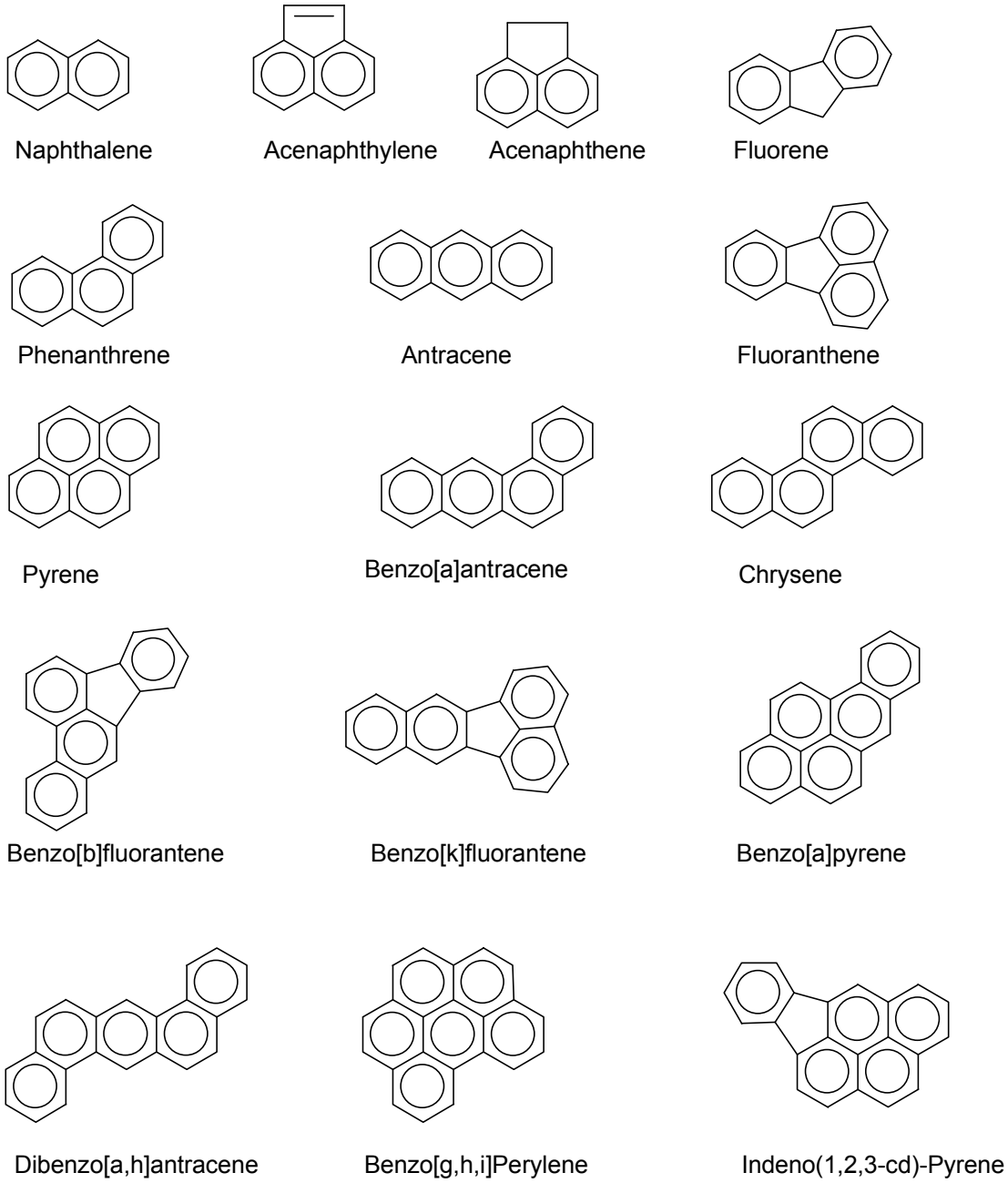


Figure 2.1: PAH compounds monitored in this study

Table 2.5: Summary of short-term toxicity tests following oral administration for a number of PAH (EC, 2002)

Compound	Species	Duration	Critical effect	NOAEL	Reference
Acenaphthene	mouse	90 days	liver toxicity	175 mg/kg bw	US EPA 1989
Anthracene	mouse	90 days	None	1000 mg/kg bw (highest dose)	US EPA 1989
Benzo[*a*]pyrene	rat	90 days	liver weight	3 mg/kg bw	Kroese *et al*., 2001
Fluoranthene	mouse	13 weeks	liver/kidney toxicity	125 mg/kg bw	US EPA, 1988
Fluorene	mouse	13 weeks	organ weight, hematology	125 mg/kg bw	US EPA, 1989
Naphthalene	mouse	90 days	None	53 mg/kg bw	Shopp *et al*., 1984
Pyrene	mouse	13 weeks	kidney toxicity	75 mg/kg bw	US EPA, 1989

NOAEL: No Observed Adverse Effect Level

Table 2.6: Sources of BaP and routes of exposures (Lioy & Greenberg, 1990)

Source	Exposures
• Foods	• Human can ingest traces of BaP in smoked, grilled, barbequed or burned foods, as cooking food at high temperatures, or charring or burning of foods increases the amount of BaP in such foods.
• Tobacco smoke	• Human can inhale BaP when they are exposed to secondhand tobacco smoke where BaP particles from cigarette smoke can cling to household dust.
• Outdoor air pollution	• Sources of BaP in outdoor air include soot, smoke and dust from the burning wood, coal, agricultural waste, incinerator emissions, and exhaust fumes from motor vehicles and other gasoline and diesel engines.
• Drinking water	• BaP primarily enter tap water by leaching from coal tar and asphalt linings in water storage tanks and distribution lines.

For example, a Dutch “market basket” study of dietary components for 18-year-old males revealed that all the PAH priority pollutants were detected. The most frequently occurring PAH were benzo(b)fluoranthene, fluoranthene and benzo(k)fluoranthene, in 59%, 48% and 46% of the samples studied, respectively. The highest concentration of a single PAH was chrysene, 36µg/kg in sugar and sweets. The mean daily intake of the total PAH fraction analyzed was between 5 µg/day (low estimates) and 17 µg/day (high estimates). The largest contribution to the daily PAH intake came from sugar and sweets, cereals, oils, fats and nuts (De Vos, 1990).

Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during heating processes such as direct fire drying, where combustion products may come into contact with the oil seeds or oil (Speer *et al.*, 1990; Standing Committee on Foodstuffs, 2001).

2.1.3 Organochlorine Pesticides (OCP)

Organochlorine pesticides are now largely banned in Malaysia. However, with their highly persistence property, their residues are still found in the environment and food products (Racke, 1993). Over the past decade, public concern over pesticide residues has risen. It is now becoming a significant food safety issue (Rekha *et al*., 2006).

Organochlorine compounds are generally not soluble in water but are very soluble in lipids. For this reason, they tend to be stored in animal tissues at levels that would depend on the intake and the metabolic peculiarities of the species concerned as well as the fat content of the species. Some aquatic organisms may acquire levels of organochlorine compounds exceeding 10 000 times of the concentration in the water they live (IARC, 1974). An additional property associated with their low solubility in water is a tendency to adsorb onto suspended particulate matter in water, on bottom sediments and on organic matter in soil. Figure 2.2 shows organochlorine compounds monitored in this study.

In the general population, the residual content of organochlorine pesticides is probably derived mainly from the diet. Other sources are from the immediate environment, such as occupational exposures, domestic uses, thermal vaporizers or public health uses. This is particularly true in the cases of DDT and HCH. Table 2.7 shows uses or sources of major organochlorine pesticides.

The primary concern for the presence of pesticide residues in the environment arise from their toxicity to all living organisms. Hence, in addition to the intended target pests, non-target organisms including humans are exposed to the toxic effects of pesticides, which may include mutagenic and carcinogenic effects.

Table 2.7: Uses of organochlorine pesticides (USEPA, 2002)

OCP	**Use / Source**
Aldrin and dieldrin	Insecticides used on crops such as corn and cotton; also used for termite control
Chlordane	Insecticide used on crops, including vegetables, small grains, potatoes, sugarcane, sugar beets, fruits, nuts, citrus and cotton. Used on home lawn and garden pests. Also used extensively to control termites
DDT	Insecticide used on agricultural crops, primarily cotton and insects that carry diseases such as malaria and typhus
Endrin	Insecticide used on crops such as cotton and grains; also used to control rodents
Heptachlor	Insecticide used primarily against soil insects and termites. Also used against some crop pests and to combat malaria

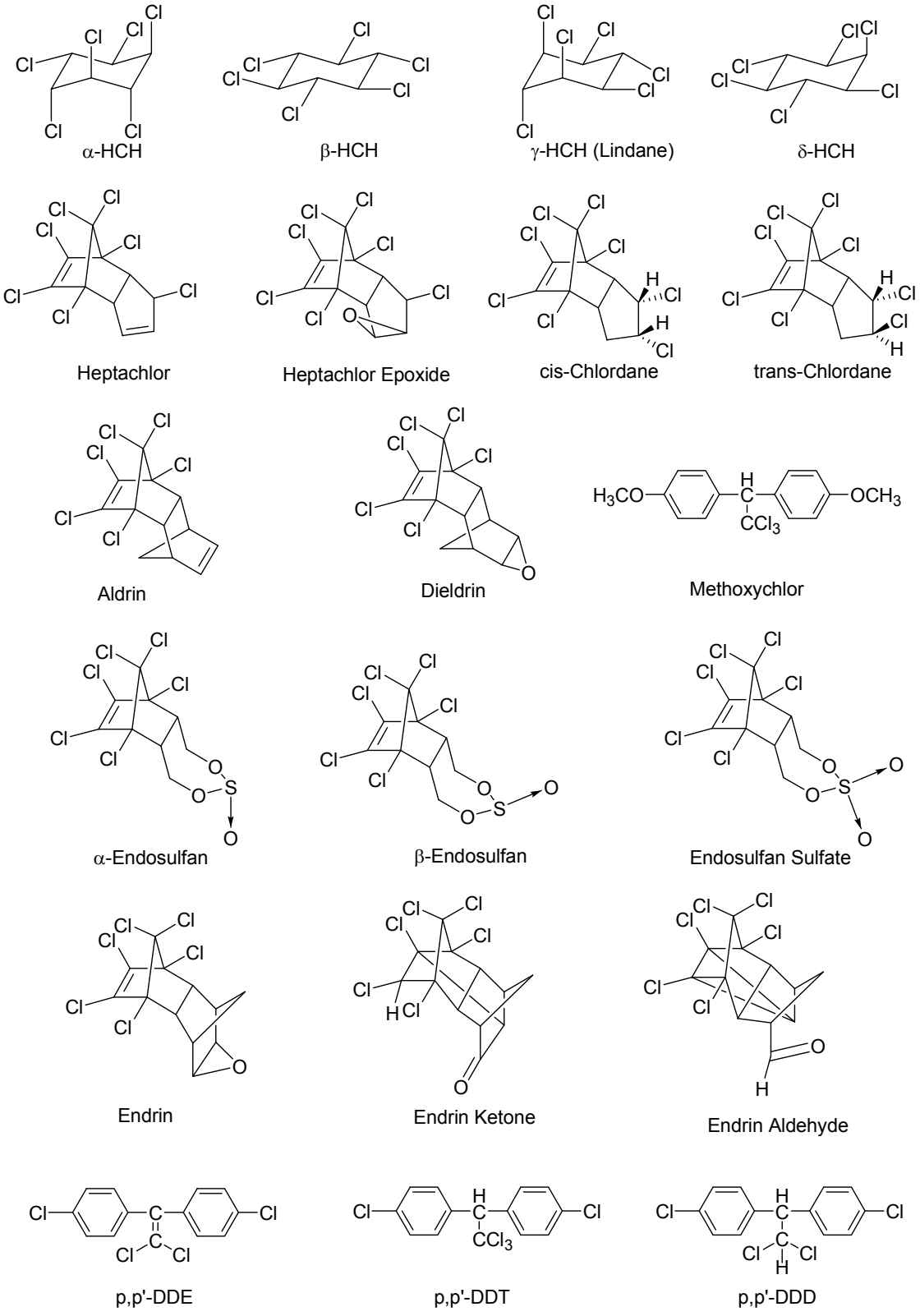


Figure 2.2: OCP compounds monitored in this study

Table 2.8 shows toxicity of OCP towards fishes and rats. Other effects, particularly bioaccumulation in the food chain are mainly associated with the organochlorine (OCP) class of compounds.

In a study by Tan and Vijayaletchumy (1994a), hexachlorocyclohexane (HCH), DDT, heptachlor and dieldrin were found in the water of many rivers flowing through rice-growing areas of Malaysia. HCH, heptachlor, aldrin and endosulfan were also detected in sediments taken from a rice growing area in Bernam and Selangor rivers (Tan and Vijayaletchumy, 1994b). Significant levels of both HCH and endosulfan were due to the extensive usage of these chemicals in the paddy fields.

In a survey conducted by Mustafa (1997), on polished rice taken from various locations in peninsular Malaysia, heptachlor were not detected in the samples with exception of the samples taken in the state of Kedah, where concentration of 7.59 ng/g fenintrothion was found in addition to the detection of o,p-DDE, p,p'-DDE and p,p'-DDT.

Table 2.8: Toxicity of OCP towards fishes and rats (Verschueren, 1983; USEPA, 1998; WHO, 2001)

OCP	LC_{50} (Fish) mg/l	LD_{50} (Rat) mg/kg	Relative Toxicity
α-HCH	>1.4	100	Moderately toxic
Lindane	0.060	88-91	Highly toxic
Heptachlor	0.150	100-162	Moderately toxic
Aldrin	0.036	67	Moderately toxic
γ-chlordane	0.009	457-590	Moderately toxic
Endosulfan	0.010	30-110	Moderately toxic
α-chlordane	0.006	200-700	Moderately toxic
Dieldrin	0.018-0.089	46-63	Highly toxic
Endrin	0.007	7.5-17.5	Highly toxic
DDT	0.103	113	Highly toxic
Methoxychlor	0.020	6000	Lowly toxic

2.2 Edible oil as a route of POPs Contamination in human

Oils and fats, along with carbohydrates and proteins are major components of human diet. Oils provide energy, fat soluble vitamins (vitamins A, D and E), and essential fatty acids that are required for proper growth and development.

The production of edible oils such as canola, corn, cottonseed, coconut, olive, palm, peanut, safflower, soybean and sunflower oils is high throughout the world, and the consumption of these oils is increasing, especially among the lower socioeconomic groups. The relative increase in the availability of oil is higher in the developing countries than in the industrialized countries in 1970-1999, 124% and 50%, respectively (WHO, 2002). Table 2.9 shows per capita vegetable oil consumption and percent daily energy intake from edible oils in selected country. A higher consumption of edible oils over animal fats is preferable because edible oils contain much less saturated fat than animal fats, and they contain no cholesterol (Giese, 1996).

Table 2.9: Per capita edible oil consumption and percent daily energy intake from edible oils in selected country (FAOSTAT, 2004)

Country	Consumption (g / day)	% Energy intake
Argentina	32	9
Brazil	35	11
Mexico	24	7
Costa Rica	32	11
Central Africa	30	14
Congo	32	14
Gambia	47	20
India	25	10
Indonesia	27	8
Philippines	13	5
Malaysia	37	12

In Malaysia, edible oils are the main source of dietary fats, due to availability of edible oils (particularly palm oil) at low prices (Josipa, 2004). A typical Malaysian meal is commonly prepared using cooking oil. For teenager and adults, the amount of cooking oil used per person for each meal (based on daily cooking oil usage in the kitchen) is estimated to be 1 to 1½ tablespoons (15-22g). Considering two main meals of the day (lunch and dinner), breakfast and fried snacks eaten during day, the total cooking oil consumed daily can easily add up to 35-45g. This amount is actually not excessive but it reflects on how cooking oil can contribute up to two-thirds of total fat intake in the local diet (NSM, 2005).

Malaysian Food Act 1983 (Act 281) had regulated the general standard for edible oils that the wholly refined oil should not contain more than 0.15 percent (%) of water and 0.2 % of free fatty acid (expressed as the main constituent fatty acid); the wholly unrefined oil, 3 % of water and 5% of free fatty acid; and refined oil blended with unrefined oil, 0.2 % of water and 1 % of free fatty acid. The regulation also stated that, cooking oil should be edible oil used for purposes of cooking and includes the edible oil for which a standard is pre-described in regulations 190 to 207, either in its single form without blending or obtained by blending two or more edible oils.

The regulations 190 to 207 briefly based on a few factors such as specific gravity, refractive index, saponification value of potassium hydroxide, iodine value and unsaponified matter in the edible oils. In Malaysian market, there are seventeen types of edible oils which follow this regulation such as refined coconut oil, unrefined coconut oil, corn oil, cottonseed oil; groundnut, peanut or arachis oil; refined, bleached and deodorized palm oil; neutralized, bleached and deodorized palm oil; bleached and deodorized palm olein; neutralized, bleached and deodorized palm olein; refined, bleached and deodorized palm kernel oil; olive oil, rice bran oil, rapeseed or toria oil, safflower seed oil, sesame seed or gingelly oil, soybean oil and sunflower seed oil.

Contamination of edible oils with POPs is caused by the uptake of traces of pesticides during growth period of plants from surrounding bio-compartments such as

soil, air and water. Due to the poor solubility of POPs in water (Worthing & Hance, 1991), there is particular concern over the long-lasting adsorption of traces of pesticides onto colloids in the soil and over specific transportation phenomena of highly volatile and sublimable POPs in the air (Seidel *et al*., 1993). Contamination levels can be reduced by refinement of the oil (USEPA, 1999), hence also partly decomposing the content of valuable highly unsaturated fatty acids and vitamins, which is provided by cold pressing of the oil seeds such as olives and many other seeds. However, this may have to be paid for by a higher content of POPs.

2.2.1 Minimal risk levels (MRLs) of POPs in edible oils

MRL is defined as an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure (EPA, 1996). Typically, MRLs are derived for different durations of exposure (acute, intermediate or chronic) and for different routes of exposure (oral or inhalation). Inhalation MRLs are exposure concentrations expressed in units of parts per million (ppm) for gases and volatiles, or milligrams per cubic meter (mg/m^3) for particles. Oral MRLs are expressed as daily human doses in units of milligrams per kilogram per day (mg/kg/day). The MRLs serve as useful reference value in evaluating human health from exposure to substances found at hazardous waste sites.

There is no regulation on MRLs for OPP (except for chlorpyrifos), PAH and OCP in edible oils under the Malaysian Food Act 1983 – Act 281 and Regulations (Food Act, 1983). However, Agency for Toxic Substances and Registry (ATSDR) had released the MRLs for ingestion of these contaminants including consumption of OPP, PAH and OCP in contaminated foods as shown in Table 2.10.

Table 2.10: Minimal Risk Level (MRL) values for OPP, PAH and OCP intake in food (ATSDR, 2005)

OPP	Minimal Risk Levels (mg/kg/day)
Chlorpyrifos	0.003
Dichlorvos	0.004
Disulfoton	0.001
Parathion methyl	0.0007
Chlorpyrifos	0.003
PAH	**Minimal Risk Levels (mg/kg/day)**
Acenaphthene	0.6
Anthracene	10
Fluoranthene	0.4
Fluorene	0.4
Naphthalene	0.6
OCP	**Minimal Risk Levels (mg/kg/day)**
Aldrin	0.002
Chlordane	0.001
p,p'-DDT	0.0005
Dieldrin	0.0001
Endosulfan	0.005
Endrin	0.002
Heptachlor	0.0001
Hexachlorobenzene	0.008
Alpha-HCH	0.008
Beta-HCH	0.05
Gamma-HCH	0.003
Methoxychlor	0.005

2.3 Analytical methods of POPs in oil samples

For monitoring traces of organic contaminants in various lipids and oils of plant origin, highly sensitive chromatographic methods have to be employed. To obtain reliable results, the lipid-soluble contaminants have to be accurately separated from the co-extracted fatty plant material (e.g. long-chain alcohols, fatty acids and esters). This step is usually the most critical part of the analytical procedure. If the procedure is not carried out properly, sensitive GC capillaries and detectors will detect impurities resulting in false positive results.

Generally, there are three main procedures involved in the analysis of POPs in oil samples, i.e. extraction of compounds of interest from oil matrix, separation of the analytes from lipids and instrumental analysis of target analytes.

2.4 Extraction method for oil samples

Pesticide residual analysis is usually carried out using the methods based on the U.S. Food and Drugs Administration's Pesticide Analytical Manual (1994) or AOAC's Official Methods of Analysis (AOAC, 1990). The conventional methods for the analysis of pesticide residues in food such as liquid-liquid extraction, supercritical-fluid extraction (SFE) or solid-phase extraction (SPE) are time-consuming, labor-intensive and require relatively large volumes of solvents. The maximum residue limits (MRLs) for pesticide residues are normally near the limit of determination, which make them difficult to analyze because of interfering compounds in food matrix. It is therefore desirable to enhance the sensitivity and remove as much as possible any interfering impurities before instrumental analysis.

After considering several factors, liquid-liquid extraction by separating funnel is chosen as extraction procedure in this study. Difficulties in separating trace organic compound in large organic matrix limit our choice in the extraction step. However, we have optimized the extraction parameters to obtain efficient extraction. Other methods

such as solid phase extraction (SPE), solid phase microextraction (SPME) and supercritical fluid extraction (SFE) are also discussed briefly in this chapter.

2.4.1 Liquid-liquid extraction by separatory funnel

Liquid-liquid extraction is a common extraction technique for analyses of pesticide residues in oil matrix. Liquid-liquid extraction, also called solvent extraction, is a process that allows the separation of two or more compounds due to their unequal solubilities in two immiscible liquid phases. With this technique, a measured volume of sample is extracted several times with portions of a water immiscible solvent.

The solvent is chosen to selectively extract certain compounds from the sample. Solvent selection for the extraction process is important because solvents differ in their extraction capabilities depending on their polarity and the solute's chemical structure (Cusack & Glatz, 1991). The desired properties of solvents are good selectivity towards solute with no miscibility with samples matrix. However, more often, the solvent would also extract significant amount of matrix. Other factors affecting solvent selection are boiling point, density, viscosity, corrosiveness, flammability, toxicity, stability, availability and cost (Cusack & Glatz, 1991)

In the analysis of pesticide residue in oil matrix, acetonitrile and n-hexane are the most commonly used solvent. Using an appropriate ratio of n-hexane and acetonitrile, pesticide residue such as PAH, OCP or OPP can be preferentially partitioned into polar acetonitrile layer while the bulk of the lipids remain solubilized in the non-polar hexane layer (Walters, 1990).

2.4.2 Solid phase extraction (SPE)

Solid phase extraction (SPE) techniques have been developed to replace the cumbersome liquid-liquid extraction methods for the determination of organic analytes in aqueous samples (AOAC, 1990). It is an extraction method that uses a solid phase sorbent and a liquid phase eluent to isolate one, or one type, of analyte from liquid

samples. Traditional liquid-liquid extraction procedures employ a serial extraction of an aqueous sample with an organic solvent resulting in a relatively large volume of solvent that must be evaporated prior to analysis. In the SPE procedures, a solid sorbent, typically an alkyl bonded silica, is packed into a cartridge or imbedded in a disk. Commercial SPE cartridges have 1-10 ml capacities but are able to handle large volume of sample by continuous elution.

For aqueous sample, reverse phase SPE is employed to extract analytes such as pesticides using solid sorbent containing non-polar functional groups such as octadecyl (C_{18}) or octyl (C_8) bonded silica. Aqueous samples are pumped or pulled through the cartridge or disk and organic compounds in the samples interact with the non-polar functional groups on the sorbent and are effectively extracted from the sample. Organic compounds attached to the sorbent are eluted from the cartridge or disk with a small volume of organic solvent (10-20 ml) (EPA, 1994).

SPE methods have been proven to be faster and easier to perform using multipart manifold to extract several samples simultaneously. Much less organic solvent is used in the sample elution process as compared to liquid-liquid extraction. Furthermore, by using small amount of solvents, potential exposure to organic solvents is decreased dramatically with SPE.

2.4.3 Solid phase micro-extraction (SPME)

Solid phase micro-extraction (SPME), an adsorption/desorption technique invented in early 1990s at the University of Waterloo (Ontario, Canada), utilizes a small segment fused silica fiber coated with an appropriate material mounted on syringe-like device for extraction of analytes from various matrices. This technique eliminates the need for solvents or complicated apparatus for concentrating volatile or nonvolatile compounds in liquid samples or headspace, making analyte extraction and pre-concentration as a single step. Besides, SPME is compatible with any packed column

or capillary gas chromatograph or gas chromatograph with mass spectrometer system, and can be used with split/splitless or direct/packed injectors (Supelco, 1998).

In analyses of chlorinated pesticides, for example, the pesticides in environmental, food or other samples usually are extracted by liquid-liquid extraction, which takes 4 to 18 hours, or with solid phase extraction cartridges or disks, which takes 1 to 2 hours total time and 20 to 45 minutes of handling time per sample (Supelco, 1998). In addition, liquid-liquid and solid-phase extraction procedures can carry contaminants into the final sample, along with the analytes of interest, producing a high background in the analysis. In contrast, SPME is faster (15 minutes) and much less labor intensive than liquid-liquid extraction, and requires only small amounts of sample and no organic solvents (Supelco, 1998). SPME reduces interfering background in pesticide or other analyses, making analyte identification and quantification more reliable.

2.4.4 Supercritical fluid extraction (SFE)

Supercritical fluids can be used to extract analytes from samples. This technique resembles soxhlet extraction except that the solvent used is at its supercritical state, a fluid above its critical temperature and pressure.

The main advantages of using supercritical fluids for extractions is that they are inexpensive, contaminant free, extract the analytes faster and more environmentally friendly than organic solvents. Besides, SFE has high diffusion coefficients of lipids, far greater than in conventional liquid solvents. Thus, the extraction rates are enhanced and less degradation of solutes occurs.

Several studies have shown that SFE is a good replacement method for traditional gravimetric techniques. In addition, carbon dioxide, which is the most common supercritical fluid, is a nonflammable compound and devoid of oxygen, thus protecting lipid samples against any oxidative degradation.